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RESEARCH ARTICLE

Extraosseous IL-6 Transgenic Mouse Plasmacytoma Sometimes Lacks *Myc*-Activating Chromosomal Translocation

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The cellular oncogene *MYC* and plasma cell growth, differentiation, and survival factor IL-6 play critical roles in the natural history of human plasma cell neoplasms such as multiple myeloma (MM). *Myc* and IL-6 also are at the center of neoplastic plasma cell transformation in BALB/c mice that carry a human IL-6 transgene and, therefore, predictably develop plasmacytomas (PCTs). We showed previously that, much like advanced MM or human myeloma cell lines (HMCLs), in which *MYC* is frequently deregulated in *cis* because of complex cytogenetic aberrations juxtaposing *MYC* to immunoglobulin enhancers, IL-6 transgenic PCTs commonly deregulate *Myc* in *cis* by chromosomal translocation, predominantly T(12;15)(*Igh–Myc*). In this article, we show that, analogous to primary MM in which *MYC* is mostly deregulated in *trans* by signaling pathways converging at the *MYC* promoter, IL-6 transgenic PCTs sometimes develop in the absence of *Myc* translocations, thus activating *Myc* in *trans*. We present cytogenetic and molecular evidence on two IL-6 transgenic PCTs that contained overexpressed Myc protein but lacked T(12;15)(*Igh–Myc*) and two related *Myc*–deregulating translocations that juxtapose *Myc* to immunoglobulin light-chain instead of heavy-chain enhancers: T(6;15)(*Igκ–Pvt1*) and T(15;16)(*Pvt1–Igλ*). We conclude that *Myc* translocations are not strictly required for IL-6-driven PCT development in mice. IL-6 transgenic PCTs may provide a valuable model system for elucidating both *trans* and *cis* mechanisms of *Myc* deregulation of great relevance for *MYC* deregulation in human MM.

INTRODUCTION

Mouse models of human plasma cell neoplasms (PCNs), such as multiple myeloma (MM) and extraosseus plasmacytoma (PCT), are indispensable for studies on the mechanisms of neoplastic plasma cell development, genetic predisposition to plasma cell tumors, and identification and validation of new therapeutic targets. Extraosseous PCTs are rare, spontaneous neoplasms of mice but are readily induced in genetically susceptible BALB/cAn (C) mice by treatment with pristane (2,6,10,14-tetramethylpentadecane), a poorly metabolized, proinflammatory isoalkane that exhibits low toxicity (Potter et al., 1962). Intraperitoneal administration of pristane in conventionally maintained (non-SPF conditions) C mice provoked the development of inflammatory granulomas, in which peritoneal PCTs arose with an average latency of 220 days and an average incidence of 60% (Potter et al., 1964). Treatment with pristane is not required in C mice carrying a widely expressed human IL-6 transgene (C.IL-6 mice), because these mice develop PCTs "spontaneously" in secondary lymphoid tissues, including Peyer's patches, the mesenteric lymph node, and the spleen (Kovalchuk et al., 2002). IL-6 transgenic (Tg) mouse PCTs present a uniting feature with human PCNs, including MM, in which IL-6 has long been recognized as a major survival factor and, more recently, as a growth and differentiation factor in tumor precursors (reviewed in Bataille et al., 2003; Chen-Kiang, 2003; Ishikawa et al., 2003; Klein et al., 2003).

Much remains to be learned from IL-6 Tg mouse PCTs before their usefulness as a model for human PCN can be fully evaluated. One important consideration concerns the onset and mechanism of *Myc* expression during tumor development. In common with their nontransgenic peritoneal counterparts (Ohno et al., 1979; Wiener et al., 1984a), IL-6 Tg PCTs are thought to be initiated by reciprocal *Myc*-activating chromosomal translocations, typically T(12;15), which joins *Myc* at 15D1 with the immu-

Abbreviations: BL, human Burkitt lymphoma; C, BALB/c; C.IL-6 mice, BALB/c mice congenic for the IL-6 Tg; HMCL, human myeloma cell line; Ig, immunoglobulin; *Igh*, mouse Ig heavy-chain locus; *IGH*, human Ig heavy-chain locus; IL-6 Tg human, IL-6 transgene driven by the H2-L^d promoter; MGUS, monoclonal gammopathy of undetermined significance; MM, human multiple myeloma; *Myc*, mouse c-*Myc* gene; *MYC*, human c-*MYC* gene; PCN, plasma cell neoplasm; PCT, plasmacytoma; SKY, spectral karyotyping.

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noglobulin heavy-chain locus *Igh* at 12F2. In contrast to mouse PCT, activation of *MYC* in human PCN—particularly MM, which has been studied more thoroughly than any other plasma cell tumor—is not affected in *cis*, is not constitutive, and is unlikely to be an initiating oncogenic event. Instead, *MYC* appears to be activated in *trans* by cellular signaling pathways that converge at the *MYC* promoter, somehow sustaining levels of gene expression that are conducive to myelomagenesis. Chromosomal translocations that deregulate *MYC* also have been observed in MM, although rarely as the reciprocal *IGH–MYC* exchanges that are consistently found in human Burkitt lymphoma (BL; Shou et al., 2000; Avet-Loiseau et al., 2001; Fabris et al., 2003). *MYC* translocations in MM and human myeloma cell lines (HMCLs) usually present as complex nonreciprocal rearrangements that constitute late tumor progression events. These often remain obscure in G-banded karyotypes but are detectable by fluorescence in situ hybridization (FISH), spectral karyotyping (SKY), and other molecular cytogenetic methods (Bergsagel et al., 2001).

IL-6 Tg PCTs lacking *Myc*-activating chromosomal translocations but containing elevated Myc protein levels might utilize the same mechanism of *Myc* deregulation that may be utilized by human MM for deregulation of *MYC*. To identify IL-6 Tg PCTs of this sort, we karyotyped six tumors that were negative in a PCR screen of illegitimate *Igh–Myc* junctions, the molecular indications of T(12;15)(*Igh–Myc*). Southern analysis of *Myc* rearrangement, FISH, and SKY demonstrated that two of these tumors lacked T(12;15) and two closely related translocations that utilize Ig light-chain enhancers instead of *Igh* enhancers to activate *Myc*: T(6;15)(*Igκ–Pvt1*) and T(15;16)(*Igλ–Pvt1*). The two *Myc*-translocation-free tumors, referred to as PCTs 1 and 2, shared many phenotypic features with their translocation-harboring counterparts, including morphology, monoclonal Ig production, and overexpression of Myc. We concluded that IL-6 Tg PCTs sometimes develop without *Myc*-activating chromosomal translocations, providing a valuable model system for elucidating *trans* mechanisms of *Myc* deregulation that may be of great relevance for *MYC* deregulation in human MM.

MATERIALS AND METHODS

Selection of IL-6 Transgenic PCTs

PCTs developed in untreated C.IL-6 congenic mice that were derived from H2-L^d-IL-6 Tg C57BL/6 mice by introgressive backcrossing of the human IL-6 transgene onto strain C to N20. PCTs were analyzed for reciprocal *Igh–Myc* junction fragments, the molecular indicators of T(12;15), using genomic PCR methods described elsewhere (Kovalchuk et al., 2000a, 2000b). Six tumors that were found to be negative by PCR screening were propagated in vivo by transfer of tumor cells i.p. to pristane-primed C mice. All mice were bred and maintained in our conventional (non-SPF) facility on the NIH campus under animal study protocol LG-028. Mice with transplanted tumors were sacrificed at an early stage of tumor outgrowth according to NCI guidelines for the ethical treatment of laboratory animals.

Detection of T(12;15) by Southern blotting and FISH

Tumor-specific *Myc* rearrangements were detected by Southern blot hybridization as previously described (Kovalchuk et al., 2002). Briefly, genomic DNA was digested with *Kpn*I, fractionated by electrophoresis on 0.7% agarose gels, transferred onto a nitrocellulose membrane, and hybridized to a 2.2-kb *Nhe*I/*Spe*I fragment of *Myc* that included exon 2. Probes were labeled with 32P by random priming. For detection by FISH of *Igh–Myc* juxtaposition, two different labeling schemes were used. In the experiment illustrated in Figure 1D, the BAC clone for *Myc* was tagged with Cy5 (blue) using a standard nick-translation protocol (McNeil et al., 2000). The BAC clones for *Igh* were labeled with Spectrum Orange (red; *Igh6*) and FITC (green; *Igh2*). In the experiment illustrated in 1C and E, BAC clones hybridizing to *Myc* or *Igh* were labeled with biotin or Spectrum Orange, followed by detection of biotin with streptavidin-FITC (Vector Laboratories, Burlingame, CA). Images were acquired with a Leica DMRXA epifluorescence microscope equipped with a Sensys CCD camera (Roper Scientific, Tucson, AZ).

Spectral Karyotyping (SKY)

To exclude the presence of the variant *Myc*-activating translocations T(6;15) and T(15;16) in tumors with an apparent lack of T(12;15), SKY was performed as previously described (Liyanage et al., 1996). Differentially labeled chromosome-specific painting probes were hybridized simultaneously onto metaphase chromosomes. Images were acquired with a custom-designed triple-pass filter using the SpectraCube SD200 (Applied Spectral Imaging, Vista, CA) connected to an epifluorescence microscope (DMRXA, Leica Microsystems,

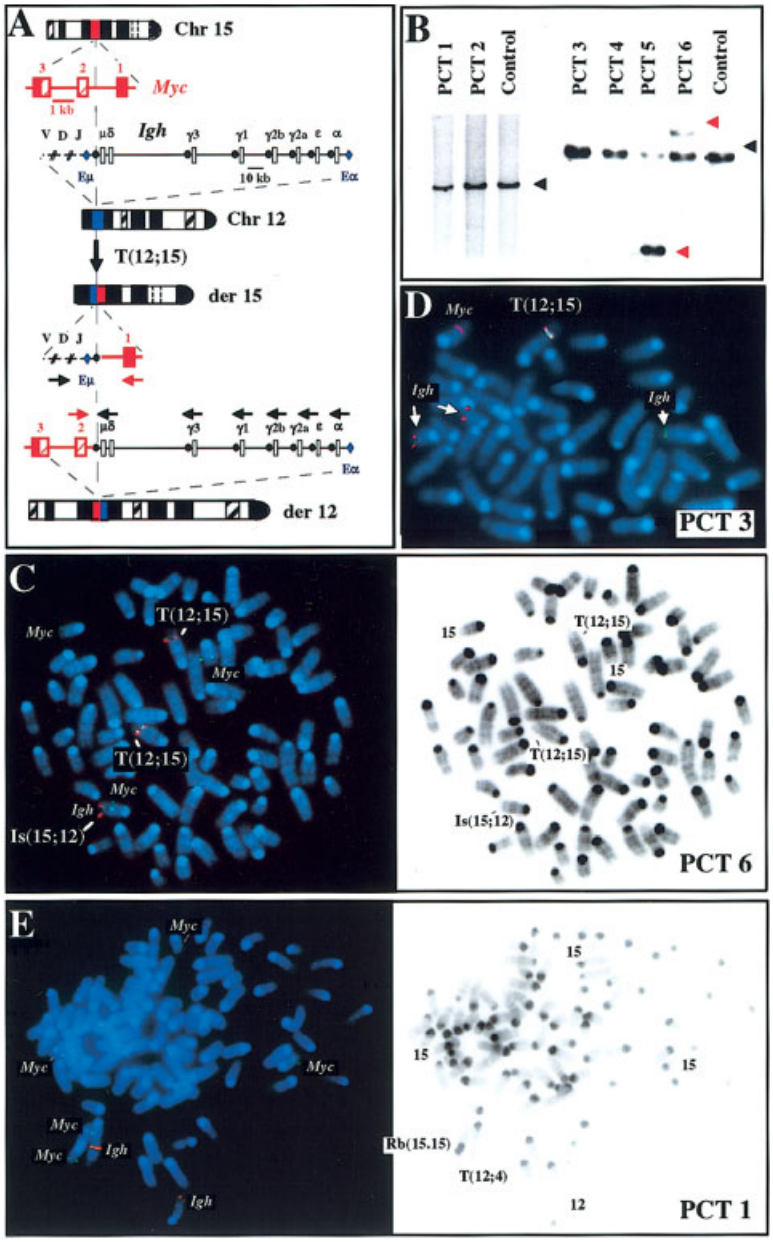

Figure 1. Detection using PCR of T(12;15) in IL-6 Tg PCTs without evidence of *Igh–Myc* junctions. (A) Scheme on the origin of balanced T(12;15) translocations and detection of reciprocal *Igh–Myc* junction fragments by use of genomic PCR. Shown at the top are ideograms of chromosomes 12 and 15 harboring *Igh* at band F2 (blue) and *Myc* at band D1 (red), respectively. The three exons of *Myc* are labeled with coding and noncoding sequences, indicated by open and filled boxes, respectively. The V, D, and J segments and the eight constant genes (C_H) of *Igh* are labeled. Switch regions are depicted as black dots to the left of the corresponding C_H. Eα and Eμ enhancers are symbolized by blue diamonds. *Myc* and *Igh* are lined up at the typical crossover point of T(12;15) in IL-6 Tg mouse PCTs: intron 1 of *Myc* and one of the switch regions, in this case, switch μ. Shown at the bottom are the reciprocal products of the T(12;15) and the location and orientation of *Myc* (red arrows) and *Igh* (black arrows) primers used for detection of reciprocal *Igh–Myc* junction fragments by PCR. *Igh–Myc* junctions cannot be detected in tumors in which the breakpoints occur far upstream of *Myc* [class III translocations according to Cory (1986)]. (B) Detection of *Myc* rearrangements in PCTs 5 and 6 by Southern hybridization. Genomic tumor DNA was digested with *Kpn*I, which restricts the *Myc* locus 3.4 kb 5′ of exon 1 and 1.7 kb 3′ of exon 3. (C) Detection of *Igh–Myc* juxtaposition by FISH in tumors that exhibited *Myc* rearrangements by Southern blotting. Shown are metaphase chromosomes of PCT 6, a near-tetraploid tumor that contains two copies of the *Myc*-deregulated product of translocation, der(12), which is visualized by colocalization of FISH probes for *Myc* and *Igh*. The tumor also contains two copies of normal chromosome 15, which harbors *Myc*. The reciprocal product of translocation, der(15), was not detected because the FISH probes used hybridized predominantly to der(12). In addition to T(12;15), the tumor contained an unusual rearrangement, Is(15;12), which was also detected by SKY (Fig. 3C). (D) Detection of *Igh–Myc* juxtaposition by FISH in tumors that showed no evidence of *Myc* rearrangements by Southern blotting. Shown are metaphase chromosomes of PCT 3, a hyperdiploid tumor that contained one copy each of der(12), containing the *Igh–Myc* fusion, and chromosome 15, containing *Myc* (purple). Three copies of chromosome 12 containing *Igh*6 (red) and *Igh*2 (green) are also present. (E) Absence of *Igh–Myc* juxtaposition in tumors that contained *Myc* in the germ-line, determined by Southern blotting. Shown are metaphase chromosomes of PCT 1, a near-tetraploid tumor that contained as many as five copies of chromosome 15 (three individual chromosomes and a Robertsonian translocation), one copy of chromosome 12 (red), and a translocation of chromosome 12 that was identified by SKY (Fig. 2A) and chromosome painting (Fig. 2C) as a nonreciprocal T(12;4). *Igh–Myc* fusion signals were not detected.

Wetzlar, Germany). A minimum of 10 SKY images and corresponding inverted 4,6-diamidino 2-phenyl-indole (DAPI) images were analyzed for each tumor according to our established protocol for analyzing mouse PCTs (Coleman et al., 1997).

Studies of IL-6 Transgenic PCTs

Histologic and immunohistochemical methods according to the criteria outlined in the Bethesda proposal for the classification of lymphoid neoplasms in mice were used for distinguishing PCTs from nonplasmacytic B-cell neoplasms (Morse et al., 2002). Serum paraproteins were detected with the help of Paragon SPE electrophoresis kits (Beckman-Coulter, Fullerton, CA). Clonotypic VDJ rearrangements were detected by Southern blot hybridization. Briefly, genomic DNA was digested with *Kpn*I, fractionated by electrophoresis on 0.7% agarose gels, transferred onto a nitrocellulose membrane, and hybridized to a 1.5-kb ^{32}P-labeled *Igh Hin*dIII/*Eco*RI probe (pJ11) that spanned JH2 and Eμ. Myc protein levels were evaluated by immunoblotting. Briefly, proteins from clarified lysates of PCTs and normal spleen (control) were resolved electrophoretically in denaturing 10% SDS-PAGE gels and transferred by electroblotting to nitrocellulose membranes. Membranes were probed with Myc antibody from rabbit (N-262, 1:500, Santa Cruz Biotechnology), followed by stripping and reprobing with actin antibody from rabbit (A2066, 1:1000, Sigma) to confirm equal loading.

RESULTS

Identification of Two IL-6 Tg PCTs That Lack T(12;15) Translocations

IL-6 Tg PCTs that do not contain T(12;15)(*Igh–Myc*) are candidates for a rare subclass of tumors that develop in the absence of *Myc*-deregulating translocations. Three such candidate tumors were identified in a previous study on plasmacytomagenesis in C.IL-6 mice (Kovalchuk et al., 2002). The study showed that the great majority of IL-6 Tg PCTs harbored T(12;15), with excellent agreement of translocation detection by cytogenetic methods (*Myc–Igh* juxtaposition using FISH) and by molecular methods (*Myc* rearrangements using Southern hybridization and *Igh–Myc* junction fragments using direct DNA PCR). Two of the three candidate tumors that, according to these methods, did not carry T(12;15) were successfully transplanted for this study and designated PCTs 1 and 2. PCR screening of *Igh–Myc* junctions (Fig. 1A) in an unreported set of 44 IL-6 Tg PCTs revealed four additional tumors with possible lack of T(12;15). These tumors also were successfully transplanted and were designated PCTs 3–6.

We performed Southern blotting of genomic DNA obtained from PCTs 1–6 in order to evaluate whether *Myc* was in the germ line, which was the expectation for tumors without T (12;15). This was the case for four of the six tumors (PCTs 1–4) but was not seen in the two remaining tumors (PCTs 5 and 6) that harbored rearranged *Myc* (Fig. 1B). We carried out a FISH analysis of *Igh–Myc* juxtaposition to assess whether the *Myc* rearrangements in PCTs 5 and 6 were caused by T(12;15). Images of metaphase cells, such as the one shown in Figure 1C, readily demonstrated that this was the case. The reason why these translocations were not detected by PCR analysis is not known. Possibilities include the deletion of primer annealing sites in *Myc* or *Igh* and complex *Igh–Myc* rearrangements such as those found in the conventional (nontransgenic), pristane-induced, peritoneal PCTs ABPC 45 (Fahrlander et al., 1985), DCPC 21 (Ohno et al., 1991), PC 7183 (VanNess et al., 1983; Shapiro et al., 1987), and MPC 11 (Greenberg et al., 1982).

FISH analysis of the four tumors that exhibited *Myc* in the germ line (PCTs 1–4) unexpectedly showed that two of them (PCTs 3 and 4) contained *Igh–Myc* juxtapositions (1D). The other two tumors (PCTs 1 and 2) lacked *Igh–Myc* juxtaposition according to FISH (1E), which was in accordance with the Southern blot data. FISH evidence for T(12;15) without *Myc* rearrangements on Southern blots suggested the presence of translocations with unusual breakpoints in the far-upstream flank of *Myc*, analogous to the *MYC*-activating t(8;14) (q24;q32) translocations in human endemic BL (Joos et al., 1992) that were designated class III translocations by Cory (1986). However, additional studies are warranted to investigate this possibility because T(12;15) translocations of class III have not been reported thus far and are not detectable by our PCR methods, which rely on primers in *Myc* coding the region in or near the 5′ flank for detection of class I/II translocations, according to Cory (1986). An alternative explanation for the *Igh–Myc* FISH signal in PCTs 3 and 4 is that an atypical T(12;15) occurred that could not be detected by Southern blotting or PCR; for example, the *Myc–Pvt1–Sα/Cα* exchange observed in tumor ABPC 60 (Shaughnessy et al., 1994).

Absence of Variant *Myc* Translocations in PCTs Without T(12;15)

Spectral karyotyping is useful for detecting "variant" *Myc*-activating translocations that may take

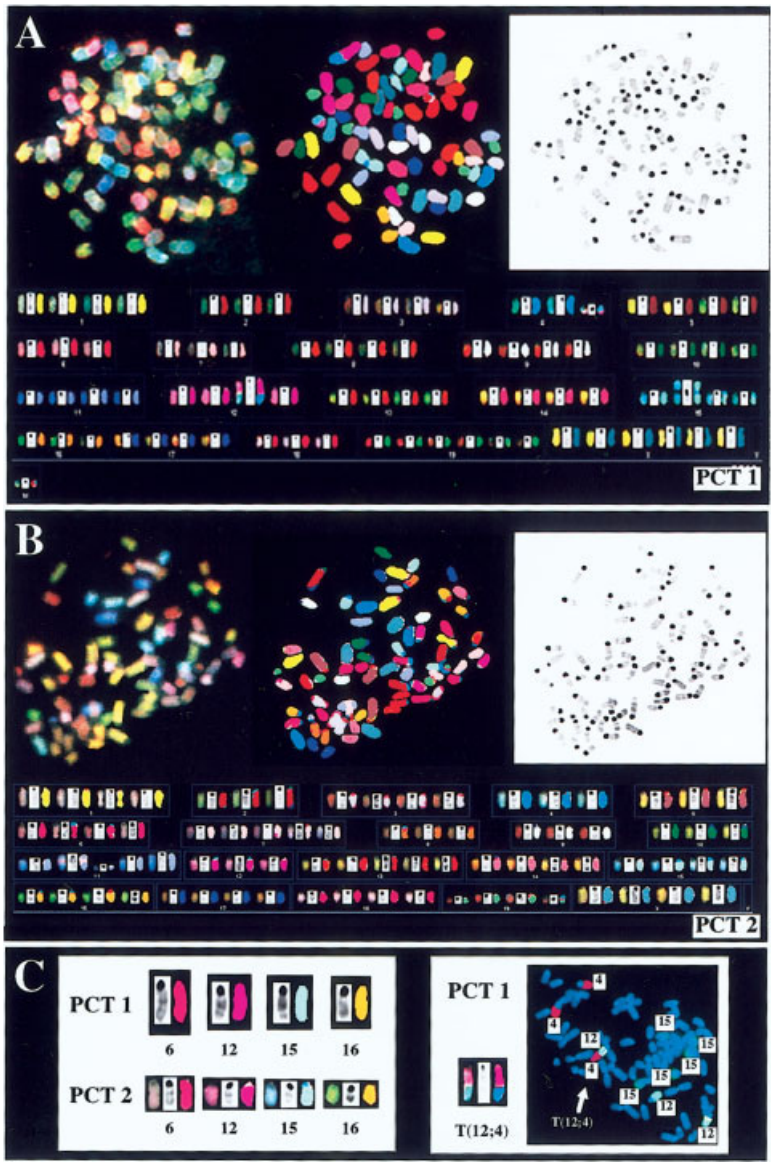

Figure 2. Spectral karyotypes of two PCTs lacking *Myc* translocations. (A) Shown at the top is a representative metaphase chromosome spread of PCT 1 in SKY display colors (left), SKY classification colors (center), and as an inverted DAPI image (right). Shown at the bottom is the complete tumor karyotype, which presents each chromosome in SKY display and classification colors and after staining with DAPI. The chromosomes are arranged in numerical order from left to right and top to bottom. A marker chromosome that could not be fully identified is shown at the bottom left. (B) Karyotype of PCT 2, presented as described above. (C) The left box illustrates that both tumors contained unchanged chromosomes 6 (*Igκ*), 12 (*Igh*), 15 (*Myc*), and 16 (*Igλ*), demonstrating a lack of *Myc–Ig* translocations. The right box depicts the SKY/DAPI images of the nonreciprocal T(12;4) found in PCT 1. The translocation was confirmed by chromosome painting presenting chromosomes 4, in red (two copies); 12, in yellow (two copies); and 15, in green (five copies). The T(12;4) is labeled in yellow and red and is indicated by an arrow.

place in PCTs lacking T(12;15). Variant translocations join the *Pvt1* locus at 15D1 (~220 kb 3′ of *Myc*) with one of the Ig light-chain loci, the *Igκ* at 6C1 or the *Igλ* at 16A3, to generate T(6;15)(*Igκ–Pvt1*), which occurs in approximately 10% of peritoneal PCTs in inbred C mice (Potter et al., 1992), or T(15;16)(*Pvt1–Igλ*), which is extremely rare (Axelson et al., 1991). SKY analysis of PCTs 1 and 2 showed that the tumors did not harbor variant *Myc* translocations (Fig. 2). Possible lack of sensitivity in detecting these translocations can be ruled out because our SKY method readily detected T(12;15) in two tumors (PCTs 5 and 6) that were included as controls because they exhibited *Igh–Myc* juxtaposition by FISH and *Myc* rearrangement by Southern blotting (Fig. 3). The modal chromosome number of the four PCTs shown in Figures 2 and 3 (79 for PCT 1, 74 for PCT 2, 45 for PCT 5, 80 for PCT 6) indicated that most tumors were hypo- or pseudotetraploid, which is typical for mouse PCTs (Potter et al., 1992).

PCT 1 contained a consistent, nonreciprocal T(4;12) that was found in 10 of 10 metaphase plates and was confirmed by whole-chromosome painting using probes for Chrs 4 and 12. A probe for Chr 15 also was included in a search for hidden rearrangements at the *Myc* locus, which were not found (Fig. 2C). PCT 2 contained a Robertsonian translocation, Rb(13.16), that was confirmed by chromosome painting (8 of 10 metaphase cells) but is not shown here because it did not involve *Igh*-, *Igκ*, or *Myc*-harboring chromosomes. PCT 6

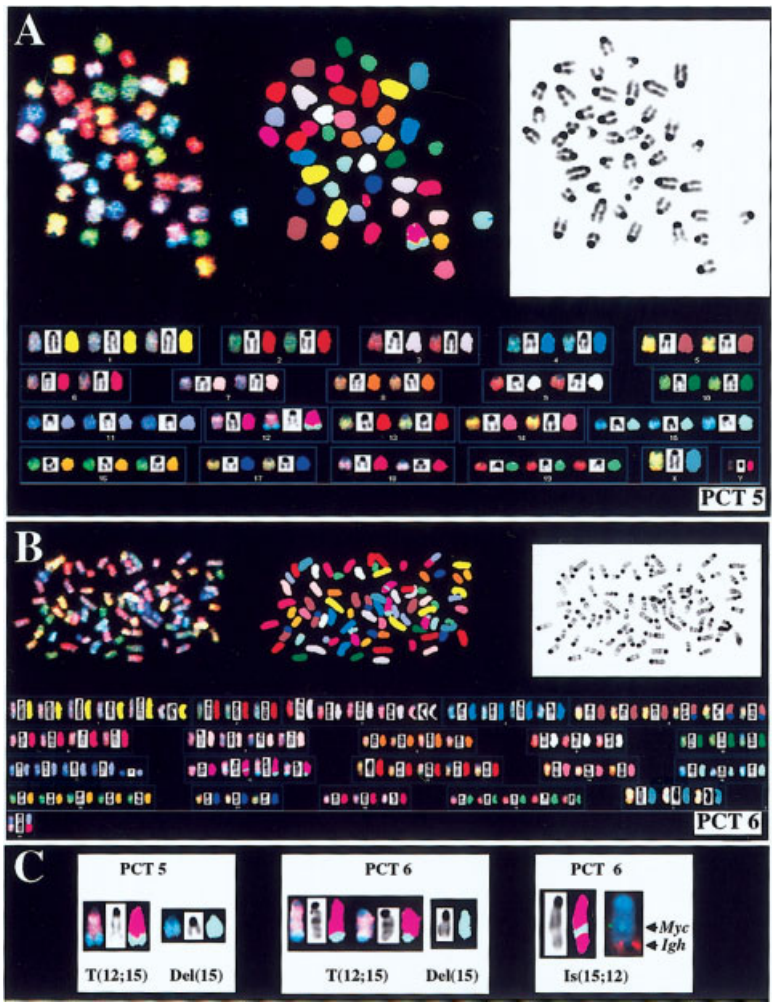

Figure 3. Spectral karyotypes of two PCTs containing T(12;15). (A) Karyotype of PCT 5, presented as described in Figure 2. This tumor was near-diploid, which is unusual for mouse plasmacytomas. (B) Karyotype of PCT 6, a near-tetraploid tumor, which is typical for mouse plasmacytomas. A dicentric marker chromosome is shown at the bottom left. (C) Shown in the left box are the reciprocal products of the *Myc–Igh* T(12;15) found in both tumors. Depicted in the right box is the SKY/DAPI image of an aberrant chromosome 12 that contained an interstitial insertion of a portion of chromosome 15: Is(15;12). The insert harbored the *Myc* locus, which was revealed by FISH (Fig. 1C) with probes for *Myc* (green) and *Igh* (red).

harbored an interstitial insertion of a chromosome 15–derived fragment into chromosome 12, indicating that SKY is sufficiently sensitive to detect aberrations that are subtler than *Myc* translocations. PCT 6 contained a T(11;6) marker chromosome and, interestingly, a nonreciprocal T(5;17) that used a previously described hot spot of rearrangements on chromosome 5 in peritoneal PCTs (Coleman et al., 2000).

The findings in PCTs 1–6 underscored the necessity of combining molecular and cytogenetic methods in order to fully clarify the translocation status of IL-6 Tg PCTs. The prevalence and types of cytogenetic changes other than *Myc* translocations found in these tumors by SKY were in line with findings in nontransgenic PCTs (Coleman et al., 2000), PCT cell lines (Coleman et al., 1997), and λ-MYC BL-like lymphomas, a recently developed mouse model of *Myc*-driven B-cell neoplasia (Kovalchuk et al., 2000c). PCTs 1 and 2, together with a previously reported subline of DCPC 21 (Wiener et al., 1999), represent the first *Myc*-translocation-free plasma cell tumors confirmed by SKY, thus extending less definitive observations in peritoneal PCTs based on G-banding (Wiener et al., 1984b; Shaughnessy et al., 1993).

Features of PCTs

Despite the differences in translocation status, PCTs 1–6 shared many phenotypic features. Histologic examination of tumor sections demonstrated that all six tumors were classic PCTs according to the recently proposed Bethesda classification of lymphoid neoplasms in mice (Morse et al., 2002). Four tumors (PCTs 1, 4, 5, and 6) displayed the mature plasmablastic/plasmacytic phenotype depicted in Figure 4A, whereas two tumors (PCTs 2 and 3) exhibited the less mature anaplastic phenotype (not shown). All PCTs produced κ light chains by immunohistochemistry (Fig. 4B). Western analysis using an anti-Myc antibody on blots of lysates of tumors showed that PCTs 2–6 expressed substan-

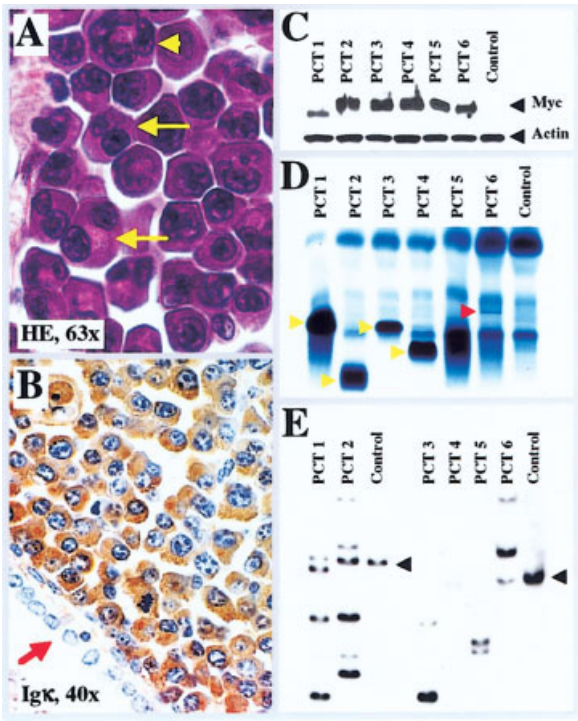

Figure 4. Features of IL-6 Tg PCTs. (A) Morphology of PCT stained with hematoxylin and eosin. Two binucleated tumor cells and an atypical tumor cell containing multiple nuclei arranged in a circle are indicated by arrows and arrowhead, respectively. (B) κ light-chain expression of a PCT that infiltrates the gut (immunostaining). Neoplastic plasma cells are indicated by brown cytoplasm. The single layer of unstained gut epithelial cells (red arrow) was negative by immunostaining. (C) Myc protein expression in PCTs 1–6 compared to spleen from C mice. Shown is an immunoblot stained with Myc antibody (top) and actin antibody, which was used as loading control (bottom). (D) Strong M-spikes in sera of mice harboring PCTs 1–5 (yellow arrowheads). Serum of mouse harboring PCT 6 contained a small M-spike that was identified by ELISA as IgM (red arrowhead). (E) Detection of *VDJ* rearrangements in PCTs 1–6 by Southern analysis. The *Igh* germ-line fragment is indicated by arrowheads.

tially elevated Myc protein levels after normalization to the actin-loading control (Fig. 4C). PCT 1 contained somewhat lower Myc levels, but these were still up-regulated compared to normal spleen, which was used as a control. Quantitative PCR by use of a TaqMan Assay-on-Demand kit for mouse *Myc* mRNA expression confirmed these results (not shown). Protein electrophoresis (Fig. 4D) demonstrated the presence of distinct M components (monoclonal Ig) in the serum of four tumor-bearing mice (indicated by yellow arrowheads) and a small but clearly visible extragradient in the mouse carrying PCT 6 (red arrowhead). Immunohistochemical stainings with μ-, λ-, and α-heavy-chain-specific antibodies and κ- and λ-light-chain-specific antibodies showed that five tumors produced IgG and one tumor (PCT 6) produced IgM (not shown), all in conjunction with κ light chains (Fig. 4B). Consistent with monoclonal Ig production, all six tumors harbored clonotypic VDJ rearrangements of the *Igh* locus (Fig. 4E). These results indicate that although IL-6 Tg PCTs comprise a relatively homogeneous group of tumors, some PCTs contain up-regulated *Myc* in the absence of *Myc* translocations.

DISCUSSION

Multiple myeloma is a human PCN that continues to be associated with high morbidity and mortality despite recent treatment advances (Barlogie et al., 2004), rediscovery of old drugs (Singhal et al., 1999), development of new drugs (Barlogie, 2003), and identification of new therapeutic targets (Hideshima et al., 2002). A large body of evidence indicates that the cellular oncogene *MYC* plays an important role in myelomagenesis, particularly during tumor progression, which is often characterized by a switch in *MYC* deregulation from *trans* (cellular signaling pathways) to *cis* (chromosomal translocations). Mouse models of human PCN driven by deregulated *Myc* may be instrumental in elucidating mechanisms of *MYC* deregulation in human MM and in devising new strategies for targeting *MYC* in order to improve therapeutic outcomes. The classic model of pristane-induced peritoneal PCT in inbred C mice (Potter et al., 1992) and the more recently developed models of accelerated PCT in Bcl-2 (Silva et al., 2003), Bcl-XL (Potter, 2003), and IL-6 (Kovalchuk et al., 2000a, 2002) transgenic C mice utilize *Myc* activation in *cis*, thus mimicking the mode of *MYC* activation seen in advanced MM. Mouse models of plasmacytomagenesis relying on *Myc* activation in *trans*, analogous to the mode of *MYC* activation seen in earlier stages of MM, are scarce, however. In this study, we showed that IL-6 Tg PCTs sometimes activate *Myc* in *trans*, providing a valuable model system for elucidating *trans* mechanisms of *MYC* activation in primary MM.

The role of *MYC* in the early stages of human myelomagenesis, including the transition from MGUS (monoclonal gammopathy of undetermined significance) to MM, is poorly defined. A highly sensitive qPCR study recently showed that only 2 of 20 (10%) MGUSs and 5 of 30 (17%) MMs expressed *MYC* (Rasmussen et al., 2003). Undetectable or low *MYC* levels in MGUS and primary MM were in accordance with the very low growth fraction of these (pre)neoplasms. Furthermore, the findings correlated well with new insights into plasma cell biology in mice, indicating that down-regulation of *Myc* and cessation of cell cycling are required for normal plasma cell development. The development of plasma cells in mice is controlled by Blimp-1 (Shaffer et al., 2002), which extin-

guishes *Myc*-driven cell growth and proliferation programs (Lin et al., 2000) and induces the cell-cycle inhibitor p18 (Tourigny et al., 2002). Plasma cell development in mice is further dependent on XBP-1, which, much like Blimp-1, suppresses *Myc* (Reimold et al., 2001; Iwakoshi et al., 2003). It is possible that Blimp-1/XBP-1-mediated repression of *MYC* is largely intact in MGUS and primary MM, but this has not been demonstrated.

Global gene expression profiling on Affymetrix microarrays has shown that *MYC* is consistently among the 50 most up-regulated genes in human MM compared to normal human bone marrow plasma cells (De Vos et al., 2002; Zhan et al., 2002). *MYC* showed eightfold overexpression in MM relative to normal plasmablasts generated in vitro from blood B lymphocytes (Tarte et al., 2003). Plasma cells from 6 of 10 (60%) MM patients exhibiting the aggressive $CD56^-$ variant of the neoplasm and 6 of 9 (67%) patients with extramedullary MM expressed significant levels of *MYC* in the qPCR study mentioned above (Rasmussen et al., 2003). Together, these findings demonstrate that *MYC* is up-regulated in *trans* during the MGUS-to-MM transition and in the early stages of MM progression. This up-regulation may involve several cellular signaling pathways that have been implicated in the proliferation, survival, adhesion, and apoptosis of myeloma cells: MAPK, PI3K, JNK/Stat3, NFκB, Scr kinase, and TGFβ (Hideshima et al., 2002; Chauhan et al., 2003; Hideshima et al., 2003). It also may lead to competition by positive transcription factors, for example, Stat3 via IL-6 signaling and E2F via MAPK signaling (Kiuchi et al., 1999), and by negative transcription factors, for example, Smad3 via TGFβ signaling (Frederick et al., 2004), for overlapping binding sites in the *MYC* promoter. Posttranscriptional mechanisms including increased translation of *MYC* mRNA from mutations in *MYC*'s internal ribosome entry site (Chappell et al., 2000), and stabilization of the MYC protein via Ras (Sears et al., 1999) and other signaling pathways (Channavajhala et al., 2002; Grumont et al., 2002), may further contribute to up-regulation of MYC protein levels in MM (Skopelitou et al., 1993; Pope et al., 1997), although many details require clarification.

Advanced MM is often characterized by a switch of *MYC* activation from *trans* to *cis* and further up-regulation of *MYC* expression. Karyotypic abnormalities in MM that involve *MYC* indicate that the switch occurs in 15% of primary and 45% of advanced tumors (Avet-Loiseau et al., 2001) and nearly 90% of HMCLs (Shou et al., 2000). Balanced *MYC* translocations, similar to those seen in BL, are, however, rarely the underlying reason (Sumegi et al., 1985; Gould et al., 1988; Hollis et al., 1988; Selvanayagam et al., 1988; Bakkus et al., 1990). Instead, most chromosomal aberrations analyzed in depth were complex nonreciprocal changes that included three-way translocations associated with deletions, inversions, duplications, and/or gene amplifications (Kuehl et al., 2002). Interestingly, many changes juxtaposed an Ig enhancer in *cis* to the *MYC* locus (Kuehl et al., 2002), indicating that MM recapitulates the outcome of BL-typical balanced *MYC* translocations by a more complicated, multistep mechanism. This suggests that the selective pressure on *MYC* overexpression is strong during MM progression. Rearrangement of *MYC* may lead to increased dependence on constitutive *MYC* expression. This is illustrated by the strict dependence of the HMCL RPMI 8226, which harbors a *MYC* insertion in a complex t(16;22) translocation, on *MYC* for growth and survival, and its undergoing apoptosis upon treatment with the cancer drug bruceantin by down-regulating *MYC* (Cuendet et al., 2004).

In summary, *MYC* may not be required in the early stages of human myelomagenesis but seems to play an important role in MM progression, in which *MYC* is initially activated in *trans*. The demand for *MYC* may increase as MM advances to a more aggressive stage, particularly the extramedullary and/or leukemic stages from which virtually all HMCLs have been derived. This may create selective pressure on myeloma cells to change *MYC* activation from *trans* to *cis*. A change of this sort also may affect the important MM oncogene *MAF*. This gene is up-regulated in approximately 40% of MMs, but this may only account for 5%–10% of the tumors by *MAF*-activating t(14;16) translocations (Hurt et al., 2004). Targeting *MYC* in MGUS and MM may provide a means to prevent the MGUS-to-MM transition and transform advanced MM into a less proliferative and less aggressive chronic disease. The availability of IL-6 Tg mouse PCTs with and without *Myc* translocations may be helpful for devising strategies for inhibiting deregulated *MYC* at different stages of MM progression.

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